DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

IN REPLYING, ADDRESS THE
Communicable Disease Center
Bacteriology Diagnostic Laboratory
P. O. Box 185
Chamblee, Georgia

November 10, 1954

Dr. Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison, Wisconsin

Dear Joshua:

I am sure that you must think me an ungrateful wretch for not having answered before now your letter in regard to our paper on anthrax bacilli. First, we wish to tell you how much we appreciate your comments, suggestions, and criticisms. We have taken them to heart and have rewritten the paper for what I believe is a great improvement. I know how much time you must have spent in the critical appraisal which you gave us. The paper has now received all the necessary security clearance from the Air Force and from Detrick so I am sending the revised copy which you may keep if you like. We will probably try to get it published as it now stands especially since Brown, who has now returned but who is taking courses here, will be transferred to California when he has finished the instruction and Gordon has left CDC employment.

Here are some of the points which you raised in regard to specific points in the paper and on the basis of which we have re-examined the data to see what further information we could obtain.

1. Question of "variable results" when transducing phage is grown on cultures susceptible to lysis other than the Ohio strain from which the phage was originally isolated. Table 1 and 2 leave much to be desired but gives a little more information on this point for they indicate that motilized strains are lysogenic for more strains than are the parent cultures although all but one of the original strains were shown to be naturally lysogenic. It is pointed out in the text that three strains of anthracis (in addition to the Ohio strain itself) which were lysed

by Ohio phage were not motilized by it. Furthermore, with one exception (19 NV) lysates of motilized cultures had no inducing activity on the parent strains. It appears that lysogenization does not always result in induction of motility and that host adaptations may occur with Ohio phage which render it unable to transduce motility to cultures formerly susceptible. Admittedly the picture is still confused and much more work needs to be done. The frequency of natural lysogenicity and the lack of suitable indicator strains specific for the Ohio phage present serious technical difficulties in making the type of genetic analysis which is needed. This is certainly the best we can do, at least for the near future.

2. I believe that it is safe to say that most motilized cultures do become lysogenic with Ohio phage based on comparisons of the presence of phage in these cultures when tests are made after 5, 32, 67 and 89 consecutive transfers of these cultures (alternate transfers consisting of plating and single colony isolation). This is brought out best in regard to culture Ax 189 (Table 2) in the parent culture of which no phage was demonstrated but which after treatment with Ohio phage was then lysogenic for all the 9 cultures on which it was tested although none of these were motilized.

3. The question of sterility testing of phages and culture purity is answered, I hope, satisfactorily in the paper as rewritten. I believe that the use of controls consisting of phage from the same container and culture from the same flask as that which resulted in motility and doing this in replicate in each experiment is adequate. All of the phage sterility tests were incubated both at 37 C and at room temperature, on and off the shaker, and for several weeks before being discarded. If spores were present which did not show up here they would have had no influence on the motile cultures, since they do not germinate under the same conditions which result in motility when the phage is present.

4. The motile variants were repurified before further testing.

5. Unmasking doesn't mean anything so we have omitted it.

6. Evidence that the induction of motility is "transduction".

   a. The Ohio strain from which the motility inducing phage was isolated (it was a naturally occurring lysogenic strain) is not motile although it is lysed by its own symbiotic phage.

   b. Not all strains which are lysed by this phage are motilized by it. Three of 10 (including Ohio) were not.

   c. When the Ohio phage is grown on some other cultures which it lyses and motilizes, it loses its ability to induce motility in cultures formerly susceptible (with 19 NV exception).

   d. The Ohio strain cannot be motilized by Ohio phage lysates of strains which have been motilized by this phage. Thus, the factors necessary for motility in Ohio strain are not present in (or carried over by phage) from any of the 6 motilized strains.

7. A marked strain would be wonderful and if we ~~could~~ do anything else with this system we should certainly mark one.

8. We have never observed a motilized culture to revert to non-motility. All have been carried in stock and have recently been retested after several transfers.

Some of the questions which you raised have not been answered since we do not have the data. I hope to find time this winter to clear up some of the questions in regard to the differentiation of transduction and lysogenization effects along the lines you mentioned. The Laboratory Branch has since my last letter to you come into possession of a Spinco so we have established (Exp. 5) that the inducing activity sediments with the phage. Of course, this still does not tell us whether or not it is a part of the phage particles.

Thanks again for a first class review and criticism of the work. We sincerely appreciated it. It seems to become increasingly hard to get anything approved for publication here as the red tape is yards thick especially if any security question is involved. It is easier to do the work than to get it ready for publication.

Dr. Joshua Lederberg--11/10/54

Please accept my best for yourself and Ester and give my regards to the bacteriologists.

Phil showed me the paper of the Japanese on O transformations in the Salmonella E. group. He thinks it too easily done to be entirely convincing, but I don't agree since this was done mainly with phage and we are accustomed to thinking of serum transformations (or perhaps anti-phage serum induced changes). Even with serum the strain of organism used seems to be the key to success at least with some of the 4, 5, 12 to 4, 12 changes in B Salmonella. With certain strains of para B its easy as pie to produce this change with 5 serum; with other one has no success at all with the same serum and conditions. Unless reversion occurs within one or two transfers the induced 4, 12 state seems to be permanent. Phil has the data on a little work we did a year or so ago.

Cordially yours,

Bill

William B. Cherry, Ph. D.
Officer-in-Charge
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you may refer to the paper in any way you should wish since it is now completely cleared.